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(71) Applicant *(for all designated States except US)*: MEDVET SCIENCE PTY. LIMITED [AU/AU]; Frome Road, Adelaide, S.A. 5000 (AU).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: LOPEZ, Angel, F. [AU/AU]; Division of Human Immunology, Hanson Centre for Cancer Research, Frome Road, Adelaide, S.A. 5000 (AU).

(74) Agent: A.P.T. PATENT & TRADE MARK ATTORNEYS; G.P.O. Box 772, Adelaide, S.A. 5001 (AU).

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(54) Title: MONOCLONAL ANTIBODY ANTAGONISTS TO HAEMOPOIETIC GROWTH FACTORS

(57) Abstract

Anti IL-3 Receptor alpha chain monoclonal antibody (MoAb) is the product of a hybridoma cell line designated 7G3. The MoAb acts as an antagonist to IL-3 in vitro activity. The MoAb binds to the N terminal domain of the IL-3 receptor alpha chain and does so competitively with IL-3 which indicates that this is, at least in part, involved in IL-3 binding. Treatment with the MoAb of fragment thereof whether recombinant or otherwise may be suitable for treating the following conditions, myeloid leukaemias, lymphomas such as follicular B cell lymphomas, or the alleviation of allergies.

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MONOCLONAL ANTIBODY ANTAGONISTS TO HAEMOPOIETIC GROWTH FACTORS

FIELD OF THE INVENTION

The present invention relates to monoclonal antibodies (MoAb) and reagents derived therefrom which are reactive with Haemopoietic Growth Factors, and especially with human interleukin 3 (IL-3) receptor α-chain and function as an antagonist to the Haemopoietic Growth Factors.

BACKGROUND OF THE INVENTION

Human interleukin-3 is a pleiotropic cytokine that stimulates the production of hemopoietic cells from multiple lineages including neutrophils, eosinophils, monocytes, megakaryocytes, erythroid cells, basophils and B cells. Recently IL-3 has also been shown to regulate vascular endothelial cell functions; enhancing adhesion molecule expression, neutrophil transmigration and cytokine production. Although some of the effects of IL-3 may be desirable and have prompted its clinical use in bone marrow reconstitution following chemotherapy, it is also apparent that abnormal or excessive production of IL-3 has the potential to lead to disease states. For example, some acute myeloid leukaemias proliferate in response to IL-3, and cells from follicular B cell lymphomas produce and depend on IL-3 for their growth. IL-3 has also been implicated in allergy not only for its ability to stimulate eosinophil and basophil production but also for being a strong stimulus of histamine release from basophils *in vitro*. The detection of elevated amounts of IL-3 mRNA in the skin and bronchi of allergic individuals further suggests an *in vivo* role in allergy.

The biological activities of human IL-3 are initiated by the binding of IL-3 to its receptor. This consists of two subunits; an α chain (IL-3Rα) which binds IL-3 specifically and with low affinity,[16] and a β chain (β_c) which does not bind ligand on its own but confers high affinity binding when co-expressed with IL-3Rα.[17,18] Both chains are required for signalling,[18] however, receptor activation and cellular signalling are dependent on IL-3 binding to IL-3Rα as the initial step. The subsequent events are not fully understood but probably involve receptor dimerization leading to the activation of specific kinases associated with the receptor.[19,43]

The structure of the extracellular domain of human IL-3Rα has not yet been elucidated. Since IL-3Rα belongs to the cytokine receptor family, it is predicted to contain a

cytokine receptor module (CRM) with two discrete folding domains.[20] In addition there is also an N-terminal domain which, interestingly, has sequence similarities with the human GM-CSF and IL-5 receptor α chains.[21] This feature distinguishes these receptors from the other members of the cytokine receptor family. The functions of the CRM and N-terminal domain of the IL-3Rα chain are not known, nor is it known where the IL-3 binding regions lie in the receptor.

SUMMARY OF THE INVENTION

The present invention arises in part as a result of the preparation and the characterization of a monoclonal antibody, MoAb 7G3, directed against the IL-3Rα chain which is capable of inhibiting IL-3 binding, IL-3 receptor dimerization[43] and of antagonizing IL-3 functions. These results offer the potential to block IL-3 activity *in vivo*. A single MoAb to hIL-3R α-chain has been previously described[35], which recognizes subpopulations of peripheral blood and bone marrow cells. However this antibody does not act as an antagonist to IL-3.

IL-3 is believed to play important roles in both haemopoiesis and inflammation. Although IL-3 has been shown to stimulate several cell types *in vitro*[37] it is somewhat puzzling that this cytokine has not been detected in the bone marrow or serum of normal animals;[38] suggesting that it is not required for basal haemopoiesis. On the other hand, the injection of IL-3 to mice and humans stimulate haemopoiesis as well as significant side-effects such as bone marrow fibrosis.[39,40] In this respect IL-3 may be viewed as a "reactive" rather than a "steady-state" cytokine and its production may lead to desirable as well as potentially deleterious effects. Consistent with this role, the production of IL-3 is under tight regulatory control in T cells.[41] We show here that MoAb 7G3 is an effective antagonist of IL-3 activities with an ED_{50} of 0.4 to 1 nM, consistent with the its K_D value (Fig 4A). Three types of IL-3 functions were studied as the antagonism of IL-3 in these situations is likely to be of clinical significance. Firstly, MoAb 7G3 antagonized IL-3-mediated enhancement of histamine release from basophils (Fig 6). Antagonizing IL-3 may be useful in allergic situations as elevated IL-3 mRNA has been noted in the skin and bronchi of atopic individuals,[15] and the presence of IL-3 may lead to excessive stimulation of basophils and eosinophils at allergic reaction sites. Secondly, the IL-3 mediated proliferation of the leukaemic cell line TF-1 was completely antagonized by MoAb 7G3 (Fig 5) at similar concentrations to those described above. Antagonism of IL-3-mediated cell proliferation is likely to be useful in some leukaemias where IL-3 has been shown to promote growth.[10,11] In particular follicular B cell lymphomas which bind IL-3 with high affinity and proliferate

in an IL-3-dependent manner[12] may be ideally suited for intervention with MoAb 7G3. Lastly, we found that MoAb 7G3 antagonized IL-3-mediated functions on HUVEC, namely the enhancement of TNF-α stimulation and the synergism with interferon γ (Fig 7). The presence of IL-3 receptors on HUVEC and their upregulation by TNF-α and interferon γ has recently been noted,[7,8,34] and their stimulation by IL-3 enhances IL-8 and IL-6 production, HLA class II expression,[8] and neutrophil transmigration.[7] Although the full significance of these *in vitro* findings need to be ascertained, these effects are likely to contribute to a systemic phase of inflammation and may be amenable to control with MoAb 7G3.

In competition experiments we found that MoAb 7G3 and IL-3 reciprocally inhibited each others binding. This suggests that the IL-3 binding site may lie within or adjacent to the epitope recognized by MoAb 7G3.

We identified the N-terminal domain of IL-3Rα as a region required for MoAb 7G3 binding based on the positive immunofluorescence and Western blotting results with a chimeric receptor comprising the N-terminal domain of IL-3Rα and the CRM[21] of GM-CSFRα. In contrast, MoAb 7G3 failed to bind to a chimeric receptor comprising the N-terminal domain of GM-CSFRα and the CRM of the extracellular region of IL-3Rα (Fig 8 and 9). This suggests that the N-terminal domain of IL-3Rα is necessary for MoAb 7G3 binding. Further truncations in the N-terminus with retention of MoAb 7G3 reactivity suggest that the 19-49 region of the N-terminal domain of IL-3Rα forms part of the epitope recognized by MoAb 7G3. In other experiments we have found that truncation of the N-terminal domain of IL-3Rα does not abolish the binding of IL-3 although the affinity of this binding is much decreased. These results have implications for defining the binding site for IL-3 and suggest that this may be formed by two non-contiguous regions in the primary structure of IL-3Rα, one of which is in the N-terminal domain and is recognized by MoAb 7G3. The existence of a conformational epitope for IL-3 and MoAb 7G3 is further supported by the inability of MoAb 7G3 to bind linear sequences as represented by the overlapping 14-amino acid peptides.

These results offer the potential to block IL-3 activity *in vivo* and suggest that the N-terminal domain of IL-3Rα, may be involved in ligand binding. An analogy may be drawn to suggest that the N-terminal domain of GM-CSFR and IL-5Rα chains, may also be involved in ligand binding because this domain is conserved amongst the IL-3Rα, GM-CSFRα and IL-5Rα family.[21]

In one broad form the invention might be said to reside in a monoclonal antibody or antibody fragment with a binding specificity to the N-terminal domain of the IL-3 receptor α chain, or of a homologous region of another haemopoietic receptor said antibody or antibody fragment capable of antagonising functions of the IL-3 or of the haemopoietic receptor respectively.

In an alternative broad form the invention could be said to reside in a monoclonal antibody or antibody fragment or any other compound, peptide or oligonucleotide, with a binding specificity to amino acid residues 19-49 of the IL-3 receptor α chain, or of a homologous region of another haemopoietic receptor.

The other haemopoietic receptor may be selected from the group of receptors responsible for binding GM-CSF, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-11, IL-13, IL-14, IL-15 and others of this family yet to be discovered, granulocyte colony stimulating factor (G-CSF), erythropoietin (EPO) and thrombopoietin (TPO), the monoclonal antibody or antibody fragment being an antagonist of the respective Haemopoietic growth factor.

In an alternative form the invention could be said to reside in a monoclonal antibody or antibody fragment being an antagonist to any one or more of the group comprising IL-3, GM-CSF and IL-5, and preferably IL-3.

As a further alternative the invention could be said to reside in a monoclonal antibody or antibody fragment being an antagonist to IL-3 activity

Alternatively the invention could be said to reside in a monoclonal antibodies or a fragment thereof produced by the 7G3 hybridoma cell line.

It is to be understood that where reference is made to a fragment of a monoclonal antibody the term to include but not be limited to Fab, Fv and peptide fragments of the monoclonal antibody, it may also include such fragments when made as part of a different larger peptide or protein, which may be the product of a recombinant vector. Thus the variable region of the respective monoclonal antibody may be cloned and be made part of a hybrid protein with properties appropriate for the therapeutic purposes of the respective agent.

The invention may also be said to reside in the method of making a monoclonal antibody, said monoclonal antibody being active as an antagonist to a haemopoietic growth factor, said method including the step of immunizing an animal with a fragment

of a haemopoietic growth factor receptor fragment including N-terminal domain of IL-3 α chain or of another haemopoietic growth factors, isolating antibody producing cells from the immunised animal and fusing said antibody producing cell with a myeloma cell to produce a pool of hybridoma cells, and screening the pool of hybridoma cells for cells that produce the monoclonal antibody.

Whilst monoclonal antibodies may be successfully used therapeutically, they are large proteins that have to be administered parenterally. Additionally they may evoke an immune response in the recipient and it is to be understood that monoclonal antibody fragments are preferably used instead of whole antibodies.

The invention therefore may reside in a therapeutic agent including a Fab, Fv or peptide fragment of a monoclonal antibody directed at amino acids 19-49 of the N-terminal domain of IL-3Rα chain, or of a homologous region of another haemopoietic growth factor receptor, said therapeutic agent acting as an antagonist to the respective haemopoeitic growth factor.

It is postulated that binding of other compounds to the same region as monoclonal antibody 7G3 or homologous regions are likely to have a similar effect. Such compounds may include peptides, oligonucleotides, amino acids, nucleic acids, or sugars. Such compounds could be isolated by screening for binding to amino acids 19-49. Accordingly the invention may be said to reside in a therapeutically active compound with a binding specificity for the N-terminal domain of IL-3Rα chain and perhaps to the amino acids 19-49 of the N-terminal domain of IL-3Rα chain, or of a homologous region of another haemopoeitic growth factor receptor, said therapeutic agent acting as an antagonist to the respective haemopoietic growth factor.

For example, a scramble of randomly synthesised oligonucleotides could be passed through a solid matrix in which a peptide of part or all of amino acids 19-49 of the N-terminal domain of IL-3Rα chain, or of a homologous region of another haemopoietic growth factor receptor. Following washing the strongly binding oligonucleotides remain and can then be eluted under different conditions (salt, ph etc). The sequence can then be determined by PCR and tested for inhibition of IL-3 on a real cell system.

The invention may also reside in a pharmaceutical preparation including the therapeutic agent, antibody, or antibody fragment defined above, in a pharmaceutically effective carrier. Such a preparation may be administered parenterally in dosage forms or formulations containing conventional nontoxic pharmaceutically acceptable carriers. Alternatively, and preferably, the agents are small enough so that they can be absorbed

via the gut, so that they may be taken orally. The formulation and preparation of any of these pharmaceutical compositions using antibodies, antibody fragments which may or may not be composed of hybrid proteins is well known to those skilled in the art of pharmaceutical formulation. Specific formulation can, however, be found in the text entitled "Remington's Pharmaceutical Sciences", Sixteenth Edition, Mack Publishing company, 1980.

It will be understood from that an antagonist of IL-3 may be useful in the treatment of leukaemias such as myeloid leukaemias, lymphomas such as follicular B cell lymphomas, or the alleviation of allergies, the invention might also be said to comprise a method of treatment or prevention of such conditions by administering a therapeutically effective dose of a therapeutic agent, or antibody, or antibody fragment as defined above in a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A Is a depiction of an Immuno-precipitation of IL-3Rα chain from ^{125}I surface-labelled F6 cells,

Figure 1B Is a depiction as for Figure 1A except showing a Western blot of F6 cells. The analyses of both Figure 1A and Figure 1B were performed on 10% SDS-PAGE under reducing conditions. The MW markers are indicated by arrows on the left-hand side of each gel.

Figure 1C Is a flow cytometry analysis of stainings of MoAb 7G3 (—) and the control MoAb (----) to COS cells transiently transfected with IL-3R α-chain, F6 cells, neutrophils, monocytes, HUVEC and eosinophils, for the experiments shown in Figures 1A and 1B.

Figure 2A. Is a graph showing dose-dependent competition for ^{125}I-IL-3 binding by MoAb 7G3 (●), 6H6 (□), 9F5 (■) and a control MoAb (○) to F6 CHO cells stably expressing the IL-3R α-chain and

Figure 2B is a graph as in Figure 2A except showing binding to COS cells transiently transfected with IL-3R α- and β-chains. In Figure 2A ^{125}I-IL-3 was used at 4nM and in Figure 2B at 150pM. In both Figures 2A and 2B The dashed line

represents competition by 200 fold excess unlabelled IL-3 and each point is the mean of triplicate determinations and the error bars represent standard deviations.

Figure 3A. Dose-dependent competition for ^{125}I-7G3 binding to F6 cells stably expressing the IL-3R α chain, and

Figure 3B is a graph as in Figure 3A except that binding is to COS cells transiently transfected with the IL-3R α and β chains by human IL-3 (●) or GM-CSF (○). In both Figures 3A and 3B the dashed line represents the inhibition in the presence of 100 fold excess unlabelled 7G3 and each point represents the mean of triplicate determinations and the error bars represent standard deviations.

Figure 4A is a Scatchard transformation of a saturation binding curve using ^{125}I-7G3 on F6 cells stably expressing the IL-3R α chain;

Figure 4B is a plot showing competition for ^{125}I -IL-3 binding to F6 cells expressing IL-3Rα chain by MoAb 7G3 (●)or IL-3 (○); and

Figure 4C is a plot showing competition for ^{125}I-IL-3 binding to COS cells expressing IL-3Rα and β chains by MoAb 7G3 (●) or IL-3 (○). For figures 4A, 4B and 4C each point is the mean of triplicate determinations.

Figure 5A is a plot showing TF-1 cell proliferation in response to different concentrations of IL-3;

Figure 5B is a plot showing TF-1 cell proliferation stimulated by 0.3 ng/ml of IL-3 in the presence of a range of concentrations of MoAb 7G3 (●), 6H6 (□), 9F5 (■) and a control MoAb (○). In figures 5A and 5B each point represents the mean of triplicate determinations and the error bars represent standard deviations.

Figure 6A Is a plot showing histamine release from human basophils in response to a range of concentrations of IL-3 and

Figure 6B Is a plot showing histamine release stimulated by 1 ng/ml of IL-3 in the presence of a range of concentrations of MoAb 7G3 (●), 9F5 (■) and the control MoAb (○). In figures 6A and 6B each value represents the mean of quadruplicate determinations and the error bars represent standard deviations.

Figure 7A. Is a representation showing that MoAb 7G3 selectively inhibits IL-3-mediated stimulation of IL-6 release from HUVEC stimulated by IL-3 (30ng/ml) together with IFN-γ (100U/ml) or TNF-α (100U/ml).

Figure 7B Is a representation showing that MoAb 7G3 selectively inhibits IL-3-mediated stimulation of IL-8 release from HUVEC stimulated by IL-3 (30ng/ml) together with IFN-γ (100U/ml) or TNF-α (100U/ml). In figures 7A and 7B MoAb 7G3 was used at 30μg/ml and the values represent the means of triplicate determinations and the error bars represent standard deviations.

Figure 8A. Is a schematic representation of the IL-3Rα constructs used to epitope map MoAb 7G3. SP = signal peptide; TM = transmembrane region; CD = cytoplasmic domain. The conserved cysteines (c) and WSXWS motifs are indicated. The numbering of the primary sequence includes the signal peptide. The shaded regions represent GM-CSFRα chain and the clear regions IL-3Rα chain encoding DNA.

Figure 8B Is a representation of the results of a Western blot analysis of COS cells transiently transfected with various IL-3Rα mutants. Binding of 7G3 was seen with the IL-3Rα/GM-CSFRα chimera (B, lane 1) and with the wild-type IL-3Rα containing a "flag" sequence interposed between the signal peptide and residue 19 (IL-3Rα flag) (B, lane 4) but not with the GM-CSFRα/IL-3Rα chimera (B, lane 2) nor with a truncated IL-3Rα lacking Thr^{19}-Asp^{49} [IL-3Rα (-31) flag] (B, lane 3).

Figure 8C Expression of IL-3Rα (-31) flag (C, lane 1) and IL-3Rα flag (C, lane 2) are demonstrated by Western Blot using an anti-flag MoAb M2.

EXAMPLES.

MATERIALS AND METHODS

Cell lines, media and cytokines. The CHO (Chinese Hamster Ovary) cell line F6, expressing the IL-3Rα chain was developed for screening and characterization of anti-IL-3Rα MoAbs. Briefly, IL-3R α-chain cDNA was cloned into pcDNA1Neo (Invitrogen, San Diego, CA) and transfected into CHO cells by electroporation.[22] COS (Monkey Fibroblast) cells transiently transfected with IL-3R α-chain cDNA by electroporation[23] with or without βc cDNA were used for immunization and characterization of anti-IL-3Rα chain MoAb. TF-1 cells were maintained in RPMI supplemented with 10% FCS and GM-CSF (2ng/ml). GM-CSF was a gift from

Genetics Institute (Cambridge, MA). Recombinant human IL-3 was produced in *E. coli* as previously described.[24]

Generation of MoAbs. BALB/c mice were immunized intraperitoneally with 5 x 10^6 COS cells transfected with the IL-3Rα chain in combination with 100μg of Adjuvant Peptides (Sigma). This procedure was repeated three times at two week intervals. Four weeks after the final immunization, a mouse was boosted intravenously with 2 x 10^6 COS cell transfectants. Three days later the splenocytes were fused with the mouse NS-1 myeloma cells at the ratio of 4:1 using 50% polyethylene glycol as described.[25] After fusion the cell suspension was cultured in RPMI-1640 supplemented with 20% FCS and 20% J774 conditioned medium.[26] Hybridoma cells were selected with hypoxanthine-aminopterin-thymidine (HAT). Hybridoma supernatants were screened using F-6 cells by an antigen capture immunoassay with Rose Bengal as a colorimetric indicator.[27] Positive clones were subcloned by limiting dilution and the culture conditions were gradually reduced to RPMI-1640 complete media supplemented with 10% FCS. Antibody-containing ascites fluid was produced by injecting the hybridoma cells into pristane-treated mice. MoAbs were purified from the ascites on a protein A Sepharose Column (Pierce, Rockford, IL) as described by the manufacturer. The MoAbs were isotyped by means of a mouse-hybridoma subtyping kit (Boehringer Mannheim, Mannheim, Germany).

Immunofluorescence. Freshly purified neutrophils, eosinophils, monocytes, human umbilical cord venular endothelial cells (HUVEC) or F6 cells ($5x10^5$) were incubated with 50μl of hybridoma supernatant or 0.25μg of purified MoAb for 45-60 min at 4°C. Cells were washed twice and then incubated with FITC-conjugated rabbit anti-mouse Ig (Silenus, Hawthorn, Victoria, Australia) for another 30-45 min. The fluorescence intensity was analysed on an EPICS-Profile II Flow Cytometer (Coulter Electronics). In experiments with truncated IL-3Rα chain and IL-3Rα/GM-CSFRα chimera, the transfected COS cells were examined under a fluorescence microscope.

Immunoprecipitations. F6 cells (4 x 10^7) were surface labelled using Na ^{125}I (New England Nuclear, Boston, MA) as described.[28] Cells were washed three times with PBS and lysed in 1 ml of RIPA buffer with protease inhibitors [25mM Tris-HCl, pH 7.4, 150mM NaCl, 1% Triton, 0.5% Deoxycholate, 0.05% SDS, 2mM PMSF, 10mM Soybean Trypsin Inhibitor, 20mM Leupeptin and 5% Aprotonin (Sigma, St Louis, MI)]. The cell extracts were centrifuged at 10,000g for 15 min and the cell lysates were precleared twice with protein A sepharose before incubating 250μl of lysates with MoAbs (2μg/ml) overnight at 4°C. Protein-A Sepharose was then added and bound proteins were washed with RIPA buffer and eluted with SDS loading buffer with 2-

mercaptoethanol and analysed by 10% SDS-PAGE. Radiolabelled proteins were visualized using an ImageQuant PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Western blotting. F6 cells (3×10^7) were solubilized in reducing SDS loading buffer, and the proteins separated by 10% SDS-PAGE before transferring electrophoretically onto nitrocellulose filters. Filters were then blocked with TNT buffer (10mM Tris-Cl pH 8.0, 150mM NaCl, 0.05% Tween-20) containing 3% gelatin. The anti-IL-3Rα MoAbs (2 μg/ml) were diluted in TNT buffer containing 1% gelatin and incubated with the filters for 90 min. The filters were then incubated with ^{125}I-Protein A (New England Nuclear, Boston, MA) for 45 min and washed thoroughly with TNT buffer. The radio-labelled proteins were detected as described above. For Western blot analysis of truncated and chimeric IL-3Rα chains, gels were electroeluted onto PVDF membrane and filters blocked in 5% BSA in Tris-buffered saline with 0.05% Tween-20 (TTBS). Filters were incubated with MoAbs (1-10μg/ml) in TTBS with 5% BSA for 2 hours. Flag tagged proteins were detected with 3μg/ml anti-flag MoAb M2 (IBI, New Haven, CT). The secondary antibody, alkaline phosphatase tagged goat anti-mouse (Pierce, Rockford, IL) was then added at a dilution of 1:2500 in TTBS. The MoAb bound proteins were visualized using the BCIP/NBT Western Blue stabilized substrate (Promega) as described by the manufacturer.

Radioiodination of IL-3 and binding assays. Radioiodination of IL-3 and binding assays were performed as previously described.[29] Briefly, low affinity binding assays were carried out by incubating 4nM of ^{125}I-IL-3 with 5×10^5 F6 cells at room temperature for $2^1/_2$ hours in RPMI medium containing 0.5% BSA and 0.1% Sodium Azide. After centrifuging through FCS, radioactivity in the cell pellet was determined by a Packard Auto-Gamma 5650 (Meriden, CT). When high affinity binding assays were performed 150pM of ^{125}I-IL-3 and 7×10^5 COS cells co-expressing IL-3R α and β chains were used. In competition experiments, cells were incubated with ^{125}I-IL-3 in the presence of a range of concentrations of MoAb 7G3 or IL-3.

Radioiodination of MoAb 7G3 and binding assays. 10μg of MoAb 7G3 was iodinated with 0.5 mCi of Na^{125}I by the Chloramine T method as described.[30] Saturation binding studies were performed by incubating 5×10^5 F6 cells with ^{125}I-7G3 over a range of concentrations (0.0018nM-20nM) in the presence or absence of 100 fold excess of unlabelled MoAb 7G3. The binding curve was analysed by Scatchard transformation.[31] In competition binding experiments F6 cells or COS cells co-expressing IL-3R α and β chains were preincubated for 2 hours at 4°C with a range of

concentrations of IL-3 or GM-CSF before adding 1nM of ^{125}I-7G3 for a further 2 hours.

Inhibition of IL-3-mediated TF-1 cell proliferation assay. TF-1 cells were starved of GM-CSF for 24 to 48 hours before setting up proliferation assays. Briefly, 1x10^4 cells were incubated in wells with 0.3ng/ml of IL-3 in the presence of a range of concentrations of MoAbs (0.00064-64 nM) for 48 hours at 37°C. Wells were pulsed with 0.5 μCi/well ^{3}H-thymidine for 4 hours and then harvested onto a glass filter and radioactivities determined by liquid scintillation. The results were expressed as disintegrations per minute (DPM).

IL-3-mediated histamine release from human basophils. Histamine release assay was determined as previously described.[14] Briefly, low density leukocytes were separated from peripheral blood by dextran sedimentation and centrifugation on Lymphoprep (Nycomed, Oslo, Norway). Cell suspension (containing 1-2% of basophils) were preincubated with purified human IgE for 45 min before incubating 1x10^6 cells with IL-3, a goat IgG anti-human IgE (0.8μg/ml) and a range of concentrations (0.00064-64 nM) of MoAbs for a further 60 min. The released histamine was quantified subsequently using a radioenzymatic method.[32]

IL-3-mediated functions on endothelial cells. The effect of MoAbs on the IL-3 stimulated secretions of IL-6 and IL-8 by HUVEC was studied. HUVEC were obtained and cultured as previously described.[33] For IL-6 measurements, HUVEC (5x10^5 per well) were treated with IFN-γ (100U/ml) for 48 hours, IL-3 (30 ng/ml) for 24 hours, or IFN-γ for 48 hours with IL-3 added for the last 24 hours with or without MoAbs 7G3 or 6H6 (100 μg/ml). After treatment the medium was changed and supernatants were collected for 24 hours and analysed for the presence of immuno-reactive IL-6 using an ELISA method (Quantikine, R & D Systems, Minneapolis, MN). IL-8 production was measured as previously described.[7] Briefly, HUVEC (5x10^5 per well) were incubated with TNF-α (100U/ml) for 24 hours and or, IL-3 (30ng/ml) for 6 hours, with or without MoAb 7G3 (50μg/ml). After incubation the medium was changed and the IL-8 secreted in the following hour was quantified by ELISA.

Construction and expression of chimeric and truncated IL-3Rα chains. IL-3Rα/GM-CSFRα chimera: this fusion cDNA encodes a chimeric receptor composed of the first 104 amino acids of IL-3Rα including the signal sequence fused to amino acids 118-400 of the GM-CSFRα-chain. It was generated by PCR using a sense primer 5' to the IL-

3Rα coding sequence and an antisense primer corresponding to codons 104-99 and including a *Kpn* 1 site. The sequence of the resulting PCR product was checked and it was then ligated in-frame to the *Kpn* 1 site at codon 118 of GM-CSFRα.

GM-CSFRα/IL-3Rα chimera: this fusion cDNA is the converse of the IL-3Rα/GM-CSFRα chimera and encodes the first 118 amino acids of GM-CSFRα-chain including the signal sequence fused to amino acids 104-378 of IL-3Rα. It was generated by PCR using a sense primer corresponding to codons 104-110 of IL-3Rα and includes a *Kpn* I site. A downstream antisense primer was also used. The resulting PCR product was sequenced and ligated in-frame to the *Kpn* I site at codon 118 of GM-CSFRα.

IL3Rα (-31) flag: this cDNA encodes an N-terminally truncated form of the IL3Rα which lacks the first 31 amino acids of the mature protein but includes an 8 amino acid "flag" peptide sequence between the putative signal sequence and residue 50 of IL-3Rα. This cDNA was generated by digesting wild-type IL-3Rα with the restriction endonuclease *Eco*R V (Boehringer Mannheim GmbH, Germany), which cleaves between codons 49 and 50 and ligating it to a PCR-generated fragment encoding the 18 amino acid signal sequence of the IL-3Rα, the "flag" sequence and a short multicloning sequence which results in Val-Asp-Asp separating the flag peptide and IL-3Rα. PCR-generated sequences were verified by DNA sequence analysis.

IL-3Rα flag: this cDNA encodes an IL-3Rα in which the putative signal sequence of IL-3Rα (first 18 amino acids) is fused to the flag peptide. It was generated by PCR using an upstream sense primer corresponding to codons 19-26 and carrying an *Xba* I site at the 5' terminus of the primer. The downstream antisense primer corresponded to codons 104-99. The resultant PCR product was ligated at the 3' end to IL-3Rα (-31) flag using a common *Bam*H 1 site to restore the coding sequence for the N-terminal 31 amino acids missing from IL-3Rα (-31) flag. The 5' end of the PCR product was ligated via the *Xba* I site to the 3' end of a PCR generated fragment encoding the IL-3Rα signal peptide followed by the "flag" sequence plus the extra amino acids Val-Asp-Asp-Ile-Ser-Arg. The fidelity of the PCR generated portion was verified by DNA sequence analysis.

All chimera and truncation constructs were cloned into the expression PMX139 prior to transfection into COS cells by DEAE-dextran. Cells were grown to approximately 50-70% confluence, washed free of medium and then incubated with 3μg of cDNA (per 10 cm plate) or 6μg of cDNA (per 15 cm plate) with 0.25 mg/ml DEAE-dextran. After

approximately 30 min the DEAE-dextran solution was aspirated off and cells washed and incubated in IMDM supplemented with 10% FCS and 100 μM chloroquine for 3-5 hours. Finally the cells were washed 3 times with serum free medium and incubated with IMDM supplemented with 10% FCS for 40-60 hours at 37°C.

RESULTS

Development of MoAb 7G3. MoAb 7G3 and other anti-IL-3Rα MoAb 6H6 and 9F5 were raised by immunizing mice with COS cell transfectants expressing the IL-3Rα chain on their surface and selecting on the stable CHO cell transfectant F6 which expresses $4x10^5$ IL-3R α chains per cell. MoAb 7G3 as well as MoAb 6H6 and 9F5 bound strongly to F6 cells (fig 1C) but not to untransfected CHO cells or CHO cell transfectants expressing GM-CSFRα chain (data not shown). To confirm biochemically the identity of the antigen identified by MoAb 7G3 as the IL-3Rα chain, immunoprecipitation and Western blot analysis were carried out. MoAb 7G3 as well as MoAb 9F5 and 6H6 specifically immunoprecipitated a protein of MW 70,000 from ^{125}I surface-labelled F6 cells whereas a control anti-GM-CSFRα chain MoAb failed to do so (Fig 1A). MoAb 7G3, 9F5 and 6H6 also recognized a protein of MW 70,000 in Western blotting of F6 cells (Fig 1B). No immunoprecipitated or Western blotted bands were seen when untransfected CHO cells were used (data not shown). Consistent with the known distribution of the IL-3R, MoAb 7G3 stained monocytes, HUVEC and eosinophils but not fresh neutrophils (Figure 1C), further confirming the identity of the antigen as the IL-3Rα chain. Identical staining was seen with 6H6 and 9F5 (data not shown). MoAb 7G3 is classified as a mouse IgG_{2a}, and 6H6 and 9F5 are mouse IgG_1.

Reciprocal inhibition of binding between IL-3 and MoAb 7G3. To examine whether the anti-IL-3Rα chain MoAb could interfere with IL-3 binding we next performed competition experiments using ^{125}I-IL-3 and cells expressing the low or high affinity IL-3 receptors. We found that MoAb 7G3 but not other MoAb inhibited the binding of ^{125}I-IL-3 to F6 cells expressing IL-3Rα in a dose-dependent manner (Fig 2A). Similarly MoAb 7G3 also blocked the binding of ^{125}I-IL-3 to COS cells transfected with the IL-3Rα and β chain cDNA (Fig 2B). In both cases, MoAb 7G3 gave 50% inhibition of ^{125}I-IL-3 binding around 0.7μM and complete inhibition around 10μM. MoAb 6H6 and 9F5 did not inhibit IL-3 binding to low or high affinity IL-3 receptors, however, 6H6 enhanced ^{125}I-IL-3 binding to the IL-3Rα chain above (Fig 2A) in 3/3 experiments performed. In reciprocal competition experiments F6 cells expressing the IL-3Rα chain alone (Fig 3A) or COS cells transfected with the IL-3Rα and β chain cDNA (Fig 3B) were preincubated with IL-3 or GM-CSF over a range of concentration

before the addition of ^{125}I-7G3. In both cases, IL-3 but not GM-CSF inhibited the binding of ^{125}I-7G3 to the IL-3R.

The affinity of MoAb 7G3 for the IL-3Rα chain Having established that MoAb 7G3 and IL-3 recognized the same or adjacent binding sites on IL-3Rα chain we next performed direct measurements of MoAb 7G3 binding and compared them to IL-3 binding. Scatchard transformation of a saturation binding curve of ^{125}I-7G3 on F6 cells revealed a K_D of 900 pM (Fig 4A). This represents a hundred fold higher affinity of IL-3Rα for 7G3 than reported for IL-3 itself.[16] Consistent with these values MoAb 7G3 competed with an approximately 100 fold greater affinity than IL-3 for ^{125}I-IL-3 binding to F6 cells (Fig 4B). On the other hand, MoAb 7G3 competed with approximately three fold lower affinity than IL-3 on COS cells expressing the IL-3 high affinity receptor (Fig 4C).

MoAb 7G3 antagonizes IL-3-mediated biological functions. Since IL-3 is a pleiotropic cytokine capable of stimulating multiple cell types and functions, we examined whether MoAb 7G3 could antagonize IL-3 functions in situations where IL-3 may play a pathogenic role, namely stimulation of cell proliferation, basophil histamine release and endothelial cell activation. To study effects on proliferation, we used the TF-1 cell line which is dependent on IL-3 for growth. A dose-response study indicated that a concentration of approximately 0.3 ng/ml of IL-3 stimulated half-maximal proliferation of TF-1 cells(Fig 5A). We found that the addition of MoAb 7G3, but not other anti-IL-3Rα chain MoAbs, to TF-1 cells stimulated with 0.3 ng/ml IL-3 antagonized cell proliferation in a dose-dependent manner (Fig 5B).

IL-3 has been shown to be one of the strongest enhancer of histamine release from human basophils, suggesting an effector role in allergy. [4,14] From a dose-response of IL-3 (Fig 6A) we selected a concentration of 1ng/ml to examine the effect of MoAb 7G3. We found that MoAb 7G3, but not MoAb 9F5, was able to completely antagonize the IL-3-dependent stimulation of basophil histamine release (Fig 6B).

Human endothelial cells have recently been shown to express IL-3 receptor α and β chain[7,34] and it has been demonstrated that IL-3 acts as an amplification factor enhancing several endothelial cell functions including cytokine secretion.[8] We found that MoAb 7G3 was able to antagonize the synergy of IL-3 with interferon γ in the stimulation of IL-6 secretion. This effect was specific for the IL-3 amplification effect and did not affect the small stimulatory effect of interferon γ alone (Fig 7A). Similarly, MoAb 7G3 was able to antagonize the enhancing effect of IL-3 on IL-8 secretion by TNF-α-stimulated cells without inhibiting the effect of TNF-α (Fig 7B).

Epitope mapping of MoAb 7G3. To identify the region(s) in IL-3Rα recognized by MoAb 7G3 we initially tested MoAb 7G3 for binding to overlapping peptides of 14 amino acids in length encompassing the full extracellular domain of the IL-3Rα chain. However, no specific binding of MoAb 7G3 was observed (data not shown). Since these results suggest that MoAb 7G3 may recognize a conformational rather than a linear epitope we generated cDNAs encoding IL-3Rα/GM-CSFRα chimeras and truncated IL-3Rα chains (figure 8A). These cDNAs were expressed in COS cells and binding of MoAb 7G3 to the mutant receptors was examined by Western blotting and immunofluorescence. Although the IL-3Rα/GM-CSFRα chimera composed of amino acids 1-104 of IL-3Rα and amino acids 118-400 of GM-CSFRα bound 7G3 by both Western blot analysis (Figure 8B) and immunofluorescent microscopy (data not shown), the converse chimera (GM-CSFRα/IL-3Rα) composed of amino acids 1-118 of GM-CSFRα and amino acids 104-378 of IL-3Rα failed to do so. This suggests that the epitope for 7G3 is located in the amino terminal 104 amino acids of IL-3Rα. A receptor deletion mutant, IL-3Rα (-31) flag, lacking the first 31 residues beyond the signal peptide (Thr19-Asp49 absent) but containing an 8 residue "flag" sequence also failed to bind 7G3. However another receptor mutant, IL-3Rα flag, containing Thr 19-Asp 49 along with the "flag" sequence did bind 7G3 (Figure 8B). Strong expression of the IL-3Rα (-31) flag and IL-3Rα flag could be demonstrated by immunofluorescent microscopy (data not shown) and Western blotting (Figure 8C) using an anti-flag M2 MoAb. These results suggest that the epitope of 7G3 may be located within amino acids Thr19-Asp49 of the amino terminus of IL-3Rα.

DEPOSIT OF CELL LINE

The cell line 7G3 was deposited on the 29th of December 1995 in the American Type Culture Collection 12301 Parklawn Drive, Rockville, Maryland, 20852, United States of America and has been designated HB-12009.

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CLAIMS

1. A monoclonal antibody or antibody fragment with a binding specificity for the IL-3 receptor α chain, said antibody or antibody fragment capable of antagonising functions of the IL-3.

2. A monoclonal antibody or antibody fragment as in claim 1 having a binding specificity to the N-terminal domain of the IL-3 receptor α chain.

3. A monoclonal antibody or antibody fragment as in claim 2 having a binding specificity to amino acid residues 19-49 of the IL-3 receptor α chain.

4. A monoclonal antibodies or a fragment thereof produced by the 7G3 hybridoma cell line.

5. A monoclonal antibody fragment as in any one of the preceding claims wherein the fragment is selected from the group comprising an Fab, Fv or peptide fragment.

6. A recombinant protein or peptide including a monoclonal antibody fragment as in claim 5.

7. A monoclonal antibody or antibody fragment with a binding specificity for the N-terminal domain of the IL-3 receptor α chain, or of a homologous region of another haemopoietic receptor, said antibody or antibody fragment capable of antagonising functions of the IL-3 or of the haemopoietic receptor respectively.

8. A monoclonal antibody or antibody fragment as in claim 7 having a binding specificity to amino acid residues 19-49 of the IL-3 receptor α chain, or of a homologous region of another haemopoietic receptor.

9. A monoclonal antibody or antibody fragment as in claim 8 wherein the other haemopoietic receptor is selected from the group of receptors responsible for binding GM-CSF, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-11, IL-13, IL-14, IL-15, granulocyte colony stimulating factor (G-CSF), erythropoietin (EPO) and thrombopoietin (TPO), the monoclonal antibody or antibody fragment being an antagonist of the respective Haemopoietic growth factor.

10. A monoclonal antibody of antibody fragment as in claim 8 being an antagonist to any one or more of the group comprising IL-3, GM-CSF and IL-5.

11. A monoclonal antibody fragment as in any one of claims 7 to 10 wherein the fragment is selected from the group comprising an Fab, Fv or peptide fragment.

12. A recombinant protein or peptide including a monoclonal antibody fragment as in claim 11.

13. A hybridoma cell line capable of producing an antibody according to any one of claims 1 to 12.

14. The hybridoma cell line 7G3 having an ATCC accession number HB-12009.

15. A therapeutically active agent with a binding specificity for the N-terminal domain of IL-3Rα chain, or of a homologous region of another haemopoietic growth factor receptor, said therapeutic agent acting as an antagonist to the respective haemopoietic growth factor.

16. A therapeutically active agent as in claim 15 with a binding specificity for amino acids 19-49 of the N-terminal domain of IL-3Rα chain.

17. The therapeutically active agent as in either claim 15 or 16 selected from the group comprising peptides, oligonucleotides, amino acids, nucleic acids, or sugars

18. A method of making a monoclonal antibody, said monoclonal antibody being active as an antagonist to a haemopoietic growth factor, said method including the step of immunizing an animal with a fragment of a haemopoietic growth factor receptor said fragment including the N-terminal domain of IL-3 α chain or the N terminal domain of another haemopoietic growth factor receptors, isolating antibody producing cells from the immunised animal and fusing said antibody producing cell with a myeloma cell to produce a pool of hybridoma cells, and screening the pool of hybridoma cells for cells that produce a monoclonal antibody that binds to a corresponding haemopoietic growth factor receptor.

19. The treatment of a condition including the step of administering a therapeutically effective dose of a therapeutic agent, or antibody, or antibody fragment as defined above in a pharmaceutically acceptable carrier, said condition selected from the group comprising myeloid leukaemias, lymphomas such as follicular B cell lymphomas, or the alleviation of allergies.

20. A pharmaceutical preparation including the therapeutic agent, antibody, or antibody fragment defined above, in a pharmaceutically effective carrier.

AMENDED CLAIMS

[received by the International Bureau on 13 May 1997 (13.05.97); original claims 1-20 amended; new claims 21-32 added; (4 pages)]

1. A method of treating a condition, comprising the step of administering a therapeutically effective dose of a monoclonal antibody or antibody fragment with a binding specificity for the IL-3 receptor α chain, said antibody or antibody fragment capable of antagonising functions of the IL-3.

2. A method of treating a condition as in claim 1 wherein the monoclonal antibody or antibody fragment has a binding specificity to the N-terminal domain of the IL-3 receptor α chain.

3. A method of treating a condition as in claim 2 wherein the monoclonal antibody or antibody fragment has a binding specificity to amino acid residues 19-49 of the IL-3 receptor α chain.

4. A method of treating a condition, comprising the step of administering a therapeutically effective dose of a monoclonal antibody or a fragment thereof produced by the 7G3 hybridoma cell line having an ATCC accession number HB-12009.

5. A method of treating a condition as in any one of the preceding claims wherein the antibody fragment is selected from the group comprising an Fab, Fv or peptide fragment.

6. A method of treating a condition, comprising the step of administering a therapeutically effective dose of a recombinant protein or peptide including a monoclonal antibody fragment as in claim 5.

7. A method of treating a condition, comprising the step of administering a therapeutically effective dose of a monoclonal antibody or antibody fragment with a binding specificity for the N-terminal domain of the IL-3 receptor α chain, or of a homologous region of another haemopoietic receptor, said antibody or antibody fragment capable of antagonising functions of the IL-3 or of the haemopoietic receptor respectively.

8. A method of treating a condition as in claim 7 wherein the monoclonal antibody or antibody fragment has a binding specificity to amino acid residues 19-49 of the IL-3 receptor α chain, or of a homologous region of another haemopoietic receptor.

9. A method of treating a condition as in claim 8 wherein the other haemopoietic receptor is selected from the group of receptors responsible for binding GM-CSF, IL-

2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-11, IL-13, IL-14, IL-15, granulocyte colony stimulating factor (G-CSF), erythropoietin (EPO) and thrombopoietin (TPO), the monoclonal antibody or antibody fragment being an antagonist of the respective Haemopoietic growth factor.

10. A method of treating a condition as in claim 8 wherein the monoclonal antibody of antibody fragment is an antagonist to any one or more of the group comprising IL-3, GM-CSF and IL-5.

11. A method of treating a conditon as in any one of claims 7 to 10 wherein the antibody fragment is selected from the group comprising an Fab, Fv or peptide fragment.

12. A method of treating a condition comprising the steps of administering a therapeutically effective dose of a recombinant protein or peptide including a monoclonal antibody fragment as in claim 11.

13. A method of treating a condition comprising the steps of administering a therapeutically effective dose of a therapeutically active agent with a binding specificity for the N-terminal domain of IL-3Rα chain, or of a homologous region of another haemopoietic growth factor receptor, said therapeutic agent acting as an antagonist to the respective haemopoietic growth factor.

14. A method of treating a condition as in claim 13 wherein the therapeutically active agent has a binding specificity for amino acids 19-49 of the N-terminal domain of IL-3Rα chain.

15. A method of treating a condition as in either claim 13 or 14 wherein the therapeutically active agent is selected from the group comprising peptides, oligonucleotides, amino acids, nucleic acids, or sugars.

16. A method of treating a condition as in any one of claims 1 to 15 wherein said condition is selected from the group comprising myeloid leukaemias, lymphomas such as follicular B cell lymphomas, or the alleviation of allergies.

17. A pharmaceutical preparation for use in treating a condition, said preparation including a monoclonal antibody or antibody fragment with a binding specificity for the IL-3 receptor α chain, said antibody or antibody fragment capable of antagonising

functions of the IL-3, said preparation also including a physiologically acceptable carrier.

18. A pharmaceutical preparation as in claim 17 wherein the monoclonal antibody or antibody fragment has a binding specificity to the N-terminal domain of the IL-3 receptor α chain.

19. A pharmaceutical preparation as in claim 18 wherein the monoclonal antibody or antibody fragment has a binding specificity to amino acid residues 19-49 of the IL-3 receptor α chain.

20. A pharmaceutical preparation for use in treating a condition, said preparation including a monoclonal antibody or a fragment thereof produced by the 7G3 hybridoma cell line having an ATCC accession number HB-12009, said preparation also including a physiologically acceptable carrier.

21. A pharmaceutical perparation as in any one of the claims 17 to 20 wherein the antibody fragment is selected from the group comprising an Fab, Fv or peptide fragment.

22. A pharmaceutical preparation for use in treating a condition, said preparation including a recombinant protein or peptide including a monoclonal antibody fragment as in claim 21, said preparation also including a physiologically acceptable carrier.

23. A pharmaceutical preparation for use in treating a condition, said preparation including a monoclonal antibody or antibody fragment with a binding specificity for the N-terminal domain of the IL-3 receptor α chain, or of a homologous region of another haemopoietic receptor, said antibody or antibody fragment capable of antagonising functions of the IL-3 or of the haemopoietic receptor respectively, said preparation also including a physiologically acceptable carrier.

24. A pharmaceutical preparation as in claim 23 wherein the monoclonal antibody or antibody fragment has a binding specificity to amino acid residues 19-49 of the IL-3 receptor α chain, or of a homologous region of another haemopoietic receptor.

25. A pharmaceutical preparation as in claim 24 wherein the other haemopoietic receptor is selected from the group of receptors responsible for binding GM-CSF, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-11, IL-13, IL-14, IL-15, granulocyte colony stimulating factor (G-CSF), erythropoietin (EPO) and thrombopoietin (TPO).

the monoclonal antibody or antibody fragment being an antagonist of the respective Haemopoietic growth factor.

26. A pharmaceutical preparation as in claim 24 wherein the monoclonal antibody of antibody fragment is an antagonist to any one or more of the group comprising IL-3, GM-CSF and IL-5.

27. A pharmaceutical preparation as in any one of claims 21 to 26 wherein the antibody fragment is selected from the group comprising an Fab, Fv or peptide fragment.

28. A pharmaceutical preparation for use in treating a condition, said preparation including a recombinant protein or peptide including a monoclonal antibody fragment as in claim 27, said preparation also including a physiologically acceptable carrier.

29. A pharmaceutical preparation for use in treating a condition, said preparation including a therapeutically active agent with a binding specificity for the N-terminal domain of IL-3Rα chain, or of a homologous region of another haemopoietic growth factor receptor, said therapeutic agent acting as an antagonist to the respective haemopoietic growth factor, said preparation also including a physiologically acceptable carrier.

30. A pharmaceutical preparation as in claim 29 wherein the therapeutically active agent has a binding specificity for amino acids 19-49 of the N-terminal domain of IL-3Rα chain.

31. A pharmaceutical preparation as in either claim 29 or 30 wherein the therapeutically active agent is selected from the group comprising peptides, oligonucleotides, amino acids, nucleic acids, or sugars

32. A pharmaceutical preparation as in any one of claims 17 to 31 wherein said condition is selected from the group comprising myeloid leukaemias, lymphomas such as follicular B cell lymphomas, or the alleviation of allergies.

FIGURE 1A

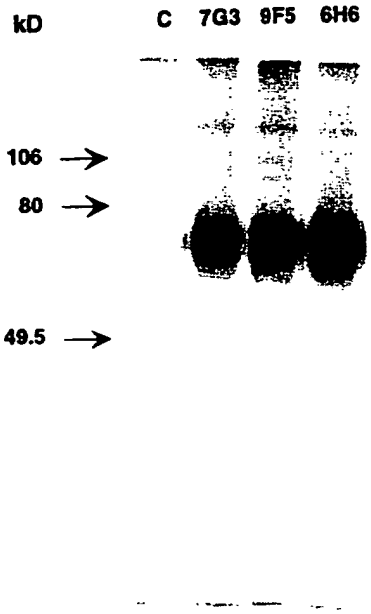


FIGURE 1B

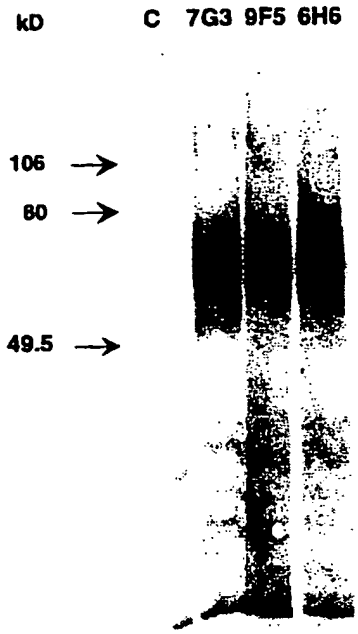
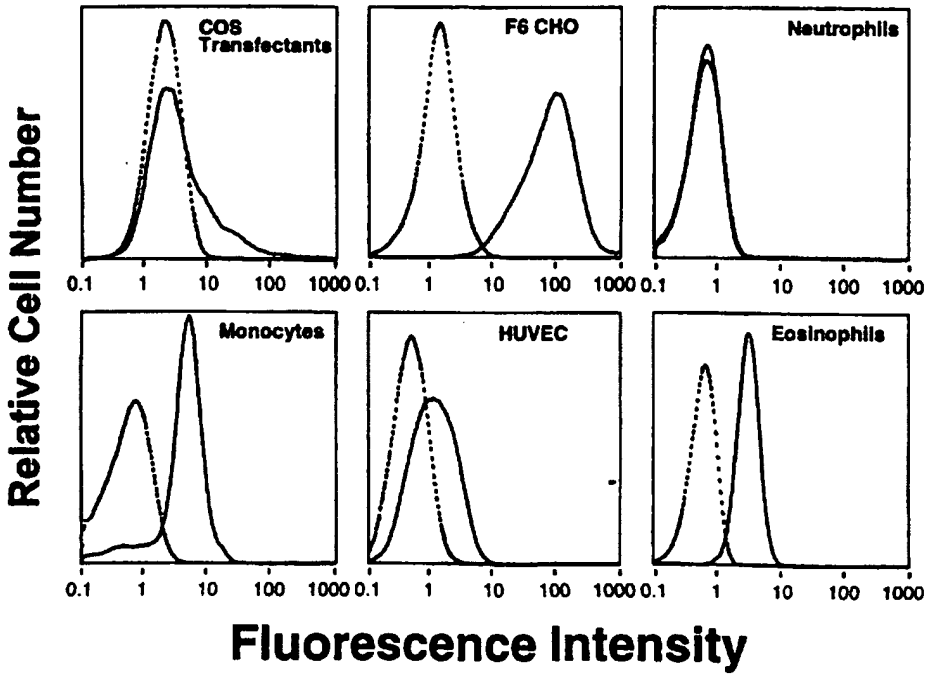


FIGURE 1C



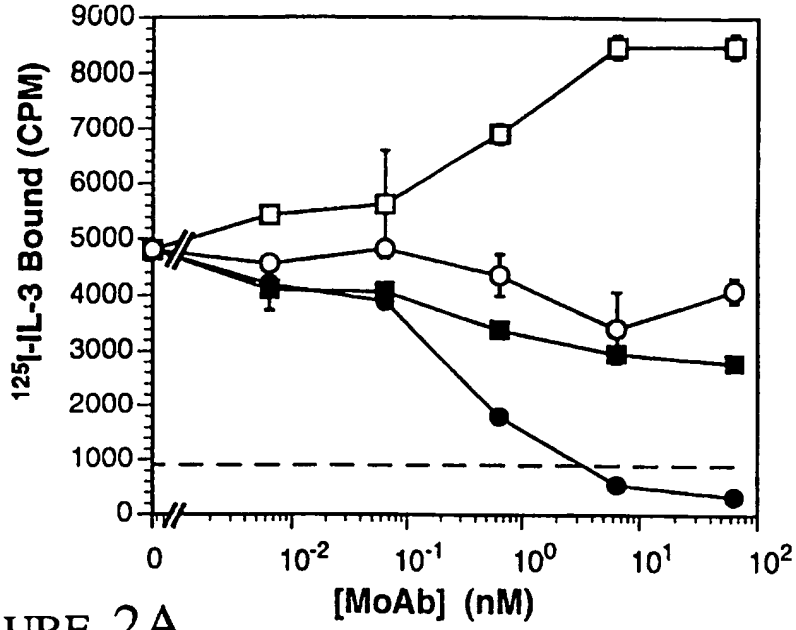


FIGURE 2A

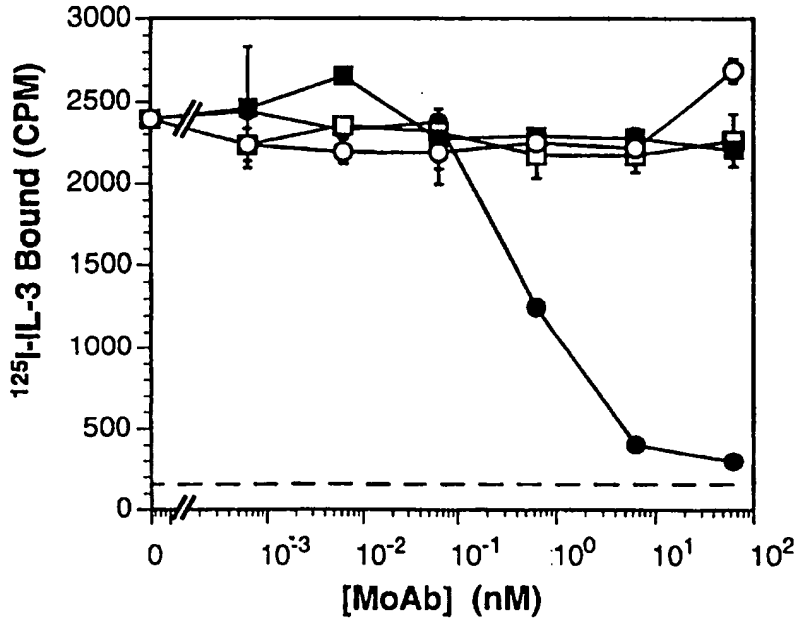


FIGURE 2B

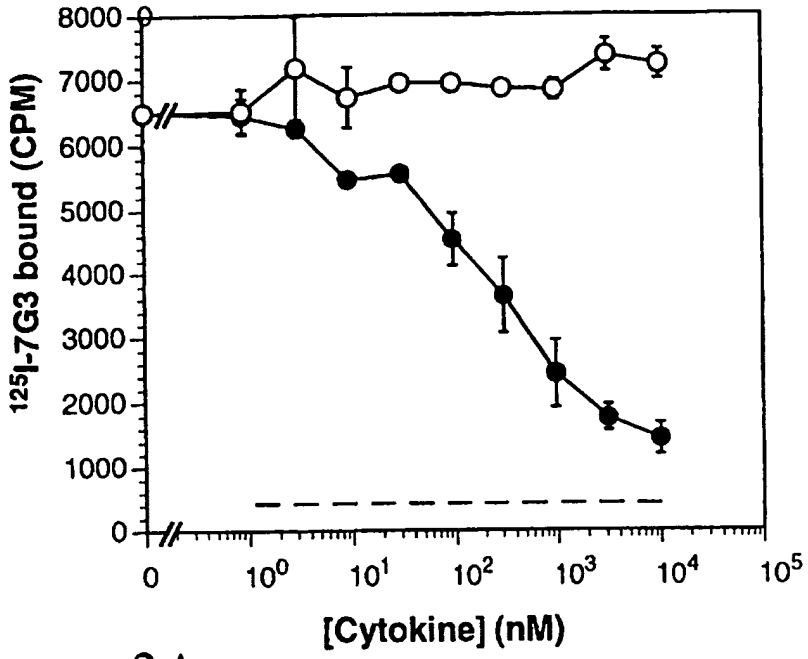


FIGURE 3A

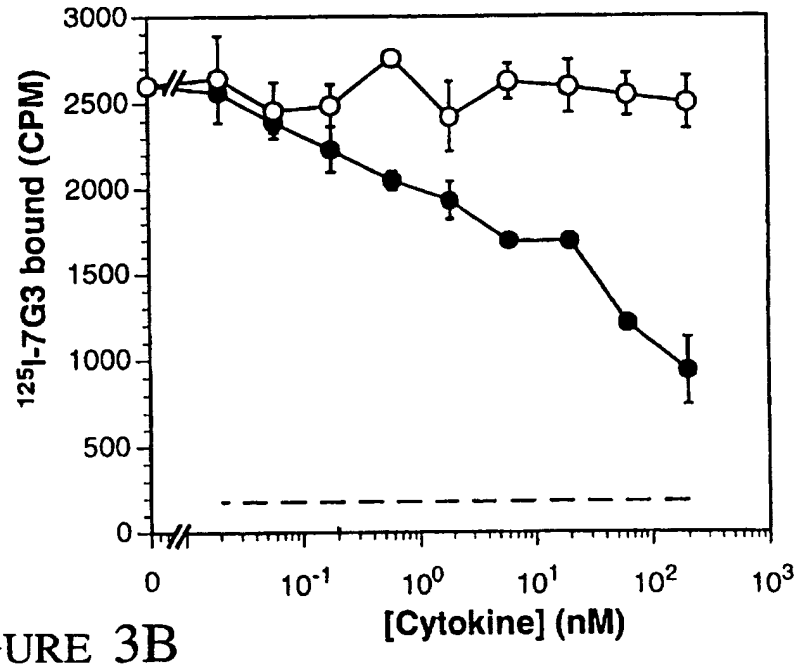


FIGURE 3B

FIGURE 4A

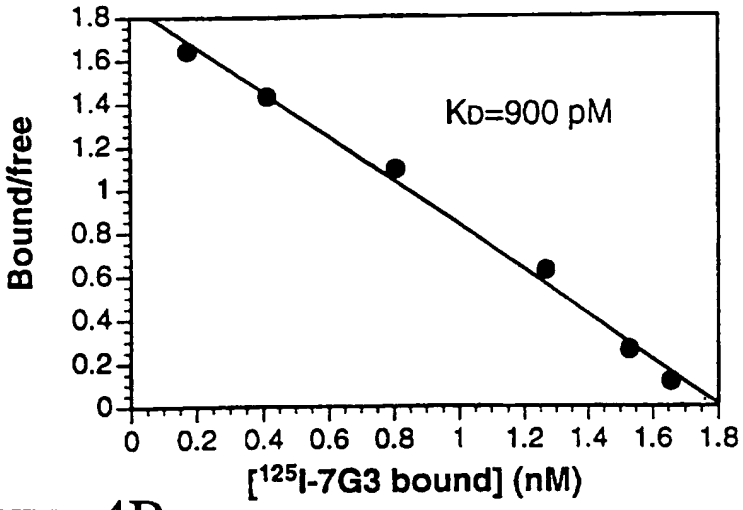


FIGURE 4B

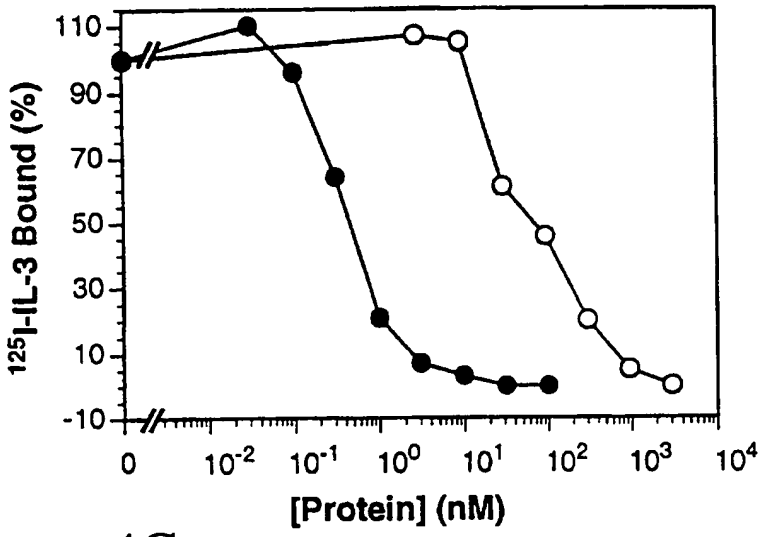
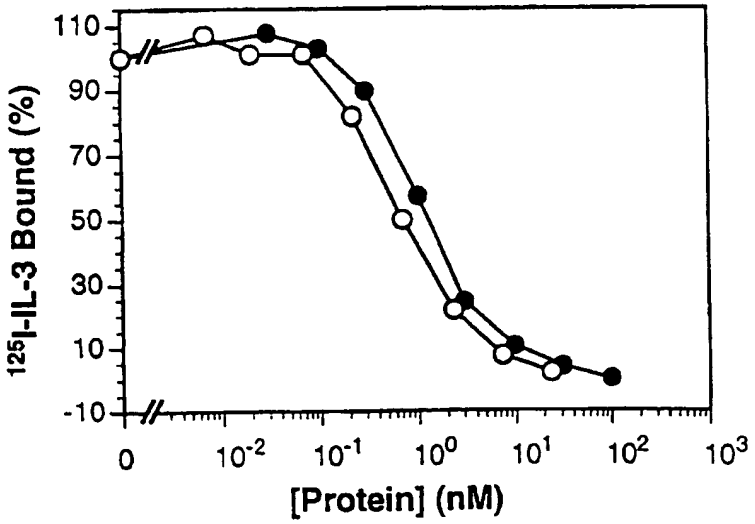


FIGURE 4C



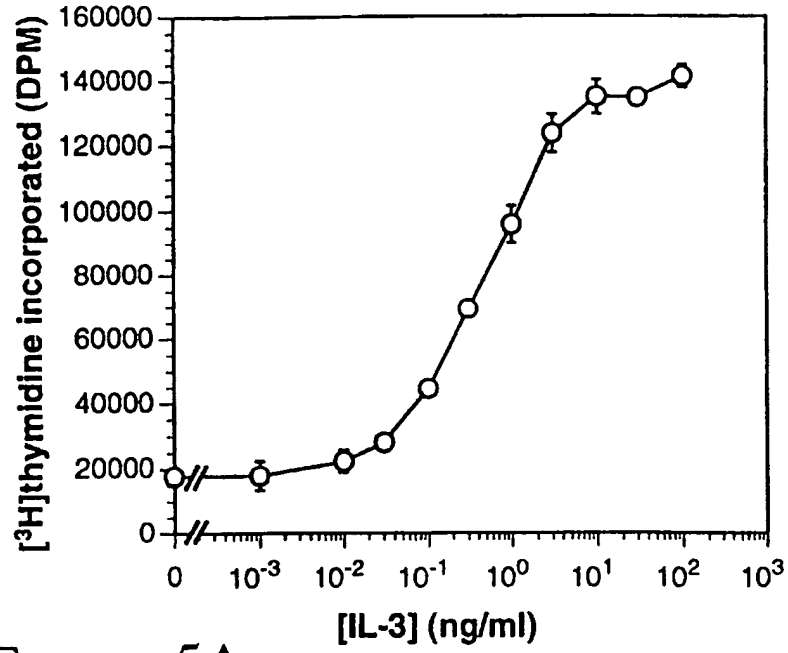


FIGURE 5A

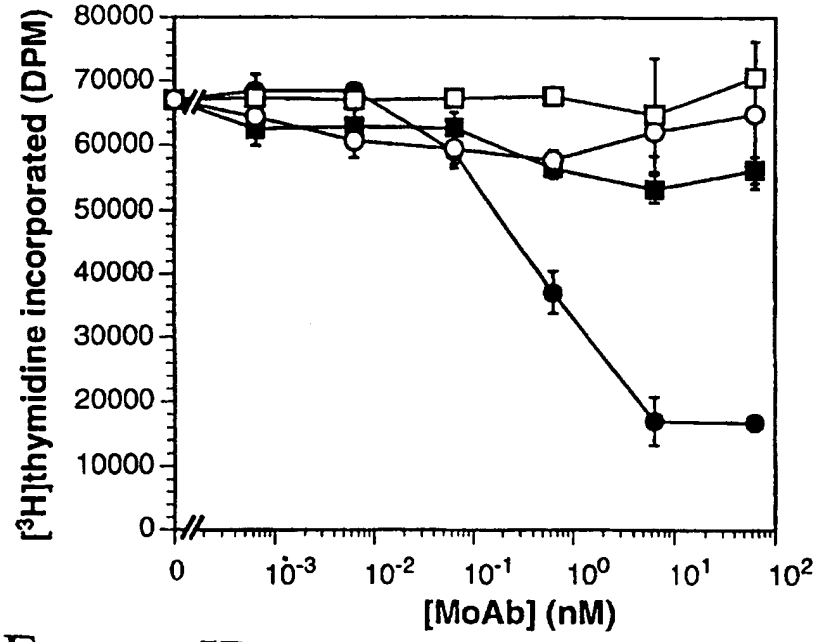


FIGURE 5B

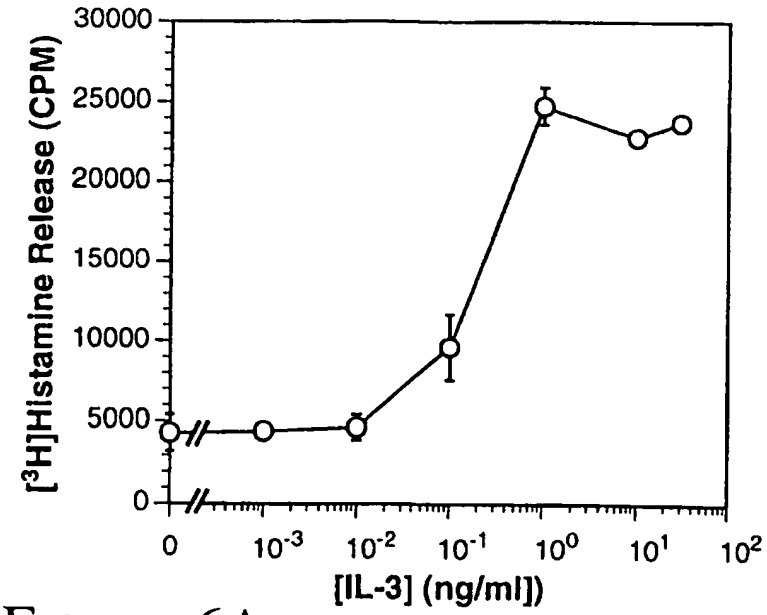


FIGURE 6A

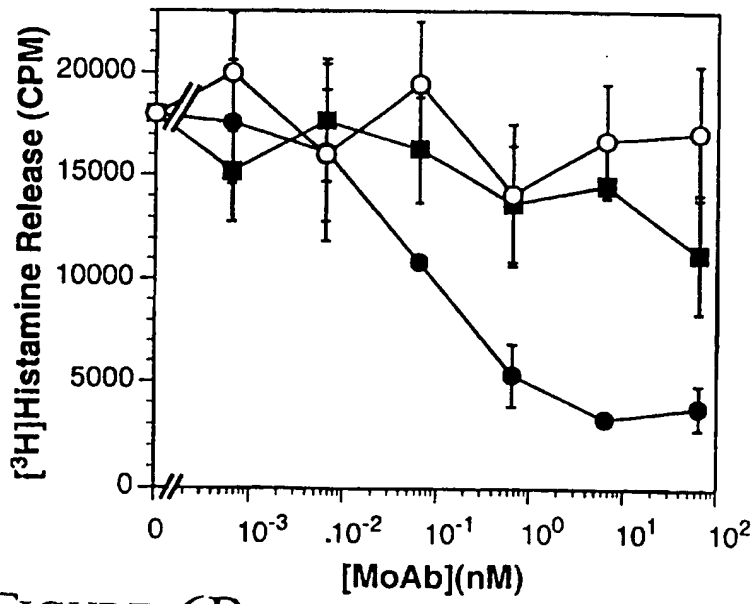


FIGURE 6B

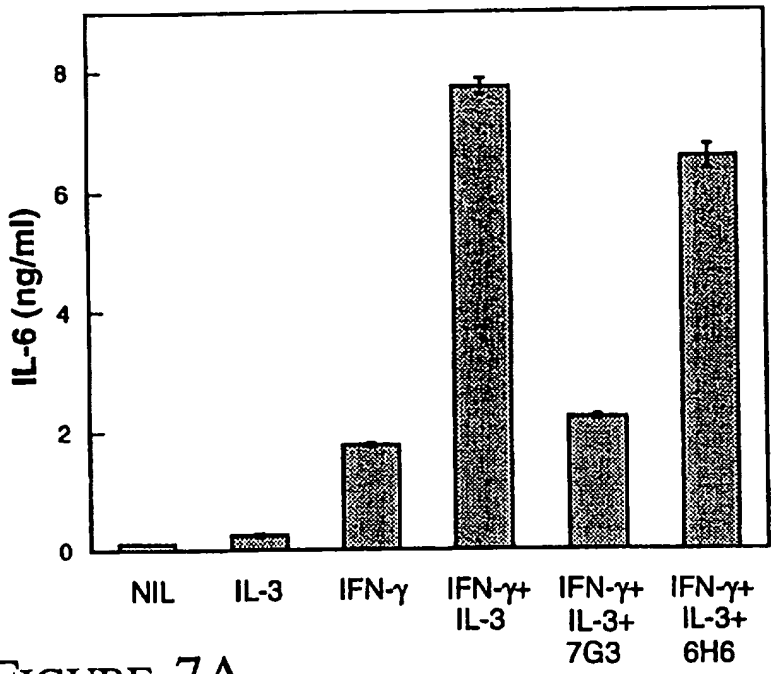


FIGURE 7A

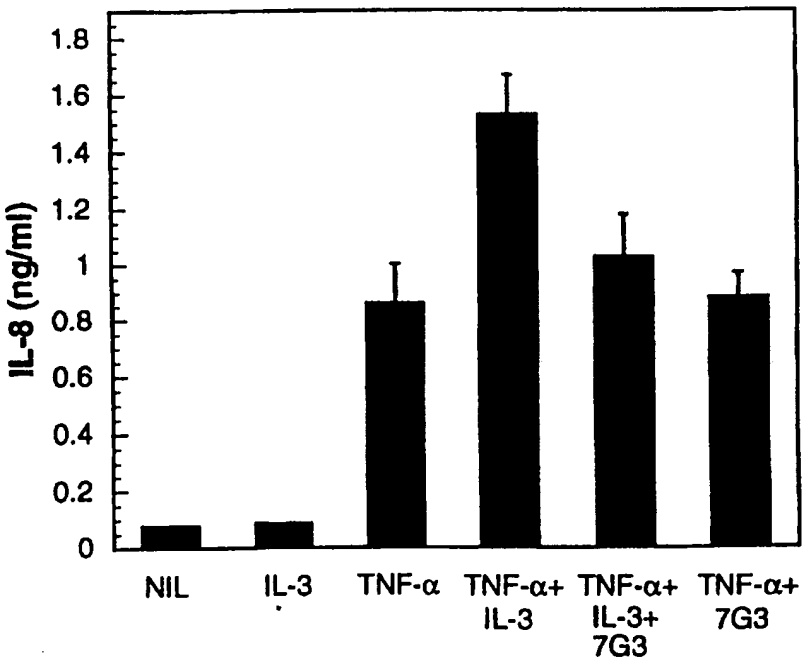
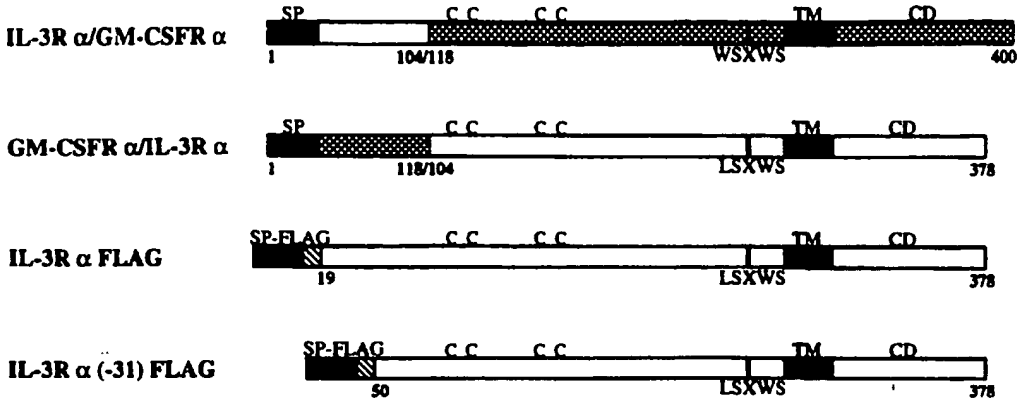
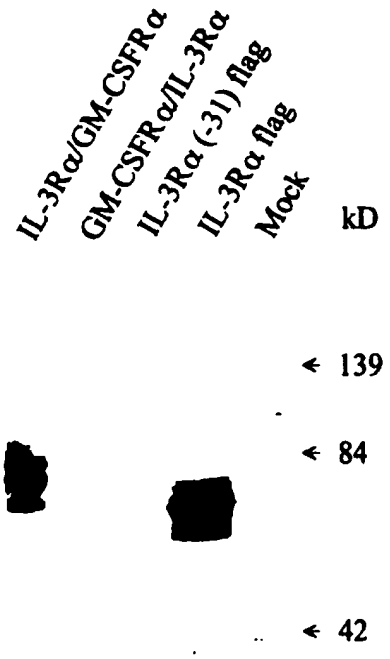


FIGURE 7B

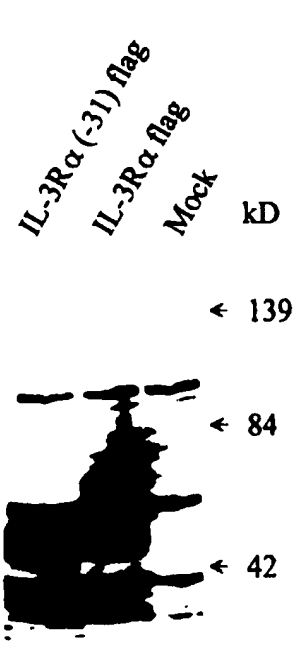
FIGURE 8A





MoAb 7G3

FIGURE 8B



Anti-flag MoAb M2

FIGURE 8C

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00840

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C07K 16/28; C12N 5/16, 5/18, 5/22; A61K 38/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IC[6]: Chemical Abstracts: keywords as below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
JAPIO: USPM: Medline: keywords as below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, JAPIO. USPM:
SS1: Interleukin (2 or 3 or 4 or 5 or 6 or 7 or 9 or 10 or 11 or 13 or 14 or 15) and receptor #

Continued

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Blood, Volume 86, No. 1, issued 1 July 1995, Korpelainen, E.I. et al., "Interferon - γ Upregulates Interleukin -3 (IL-3) Receptor Expression in Human Endothelial Cells and Synergizes With IL-3 in Stimulating Major Histocompatibility Complex Class II Expression and Cytokine Production", pages 176-182, see page 177, column 2 and page 178, column 2	1-20

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 January 1997	14 MAR 1997
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (06) 285 3929	Authorized officer **KAREN AYERS** Telephone No.: (06) 283 2503

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00840

C (Continuation) **DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of Experimental Medicine, Volume 163, No. 2, issued 1 Feburary 1986, Palacios, R. et al., "Monoclonal Antibodies Specific for Interleukin 3- Sensitive Murine Cells", pages 369-382, see pages 373-376 and 379-380	1, 2, 5-7, 11-13, 15, 17-20
X	AU, B, 51450/93 (673858) (AMRAD CORPORATION LIMITED) 9 May 1994, see Example 9 and claims	7, 11-13, 15, 17-20
Y	Journal of Leukocyte Biology, Volume 57, No.6, issued June 1995, Devos, R. et al., "Interleukin - 5 and its receptor: a drug target for eosinophilia associated with chronic allergic disease", pages 813-819, see pages 813 and 816-817	7, 11-13, 15, 17-20
Y	The Journal of Immunology, Volume 147, No. 10, issued 15 November 1991, Van der Heyden, J. et al., "Characterization of the Murine IL-5 Receptor Complex with the use of a Panel of Monoclonal Antibodies; Relationship to the Murine IL-3 Receptor", pages 3413-3418, see pages 3416-3417, discussion	7, 11-13, 15, 17-20
P,X	Blood, Volume 87, No. 1, issued 1 January 1996, Sun, Q. et al., "Monoclonal Antibody 7G3 Recognizes the N-Terminal Domain of the Human Interleukin -3 (IL-3) Receptor α-chain and Functions as a Specific IL-3 Receptor Antagonist", pages 83-92, see the entire document	1-20

Form PCT/ISA/210 (second sheet) (July 1992) copdeg

INTERNATIONAL SEARCH REPORT

...ernational Application No
PCT/AU 96/00840

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 15-17
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

It is impossible to determine and search the entire scope of the term " therapeutic binding agent" as it could include a wide range of compounds including, for example, interleukins or other haemopoietic growth factors (including variants of IL-3 itself) which act as antagonists to IL-3 receptor. As such it is impossible to arrive at a reasonable conclusion as to the scope of the claimed invention.

3. ☒ Claims Nos.: 19, 20
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Box B (Continuation) Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

SS2:Granulocyte macrophage colony stimulating factor and receptor#

SS3: Granulocyte colony stimulating factor and receptor #

SS4: Erythropoietin and receptor#

SS5: Thrombopoietin and receptor #

SS6: (SS1 OR SS2 OR SS3 OR SS4 OR SS5) and monoclonal (antibod: or ab#) or mab #

CAS online, Medline:

SS1: (Interleukin (3 or 5) and receptor #) or receptors interleukin (3 or 5).

SS2: SS1 and (monoclonal antibody or monoclonal antibodies).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.
PCT/AU 96/00840

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
AU	51450/93	WO	9409149	EP	668923

END OF ANNEX